# Sexual Dimorphism and Geographic Variation in the Asian Lance-headed Pitviper *Protobothrops mucrosquamatus* in the Mainland China

Guanghui ZHONG<sup>1, 2</sup>, Qin LIU<sup>2</sup>, Cao LI<sup>2</sup>, Peihao PENG<sup>1</sup> and Peng GUO<sup>2\*</sup>

**Abstract** Sexual dimorphism (SD) and geographic variation (GV) are widespread in snakes. *Protobothrops mucrosquamatus* (Cantor 1839) is one of the most common Asian venomous snakes with a wide geographical distribution. We examined SD and GV patterns for this species by using multivariate statistical analyses of external morphological characters scored from specimens from the Mainland China. The result displayed that SD was significant in several external characters in *P. mucrosquamatus*, and the male *P. mucrosquamatus* formed two distinct clusters (Hainan Island and mainland China), but the females did not. Based on our present work and the other data, we concluded that no significantly intraspecific differentiation is present within this species.

**Keywords** morphology, sexual dimorphism, geographic variation, venomous snake, intraspecific taxonomy, *Protobothrops mucrosquamatus*, China

# 1. Introduction

Sexual dimorphism (SD) and geographic variation (GV), which are widespread among animals, have attracted a considerable number of scientists (Campbell, 1972; Darwin, 1871; Dewitt et al., 2008; Shine, 1986). In snakes, SD and GV, involving a variety of traits such as body size, body shape, coloration, markings, and scalation, have been well documented (Aubret et al., 2005; Bonnet et al., 1998; Gregory, 2004; Malhotra and Thorpe, 2004; Shine, 1994). However, the degree and pattern of SD and GV vary in different taxonomical groups or species (see Aubret et al., 2005). For example, the female grass snakes are larger than the males in body size (Snout-ventral length), but a reverse pattern is detected in an American pitviper Agkistridon piscivorus (see Shine, 1978); Australia boa Morelia spilota reveals a different pattern of SD between Northeast and Southeast

E-mail: ybguop@163.com

Received: 31 July 2016 Accepted: 18 November 2016

groups (Pearson et al., 2002).

Protobothrops mucrosquamatus (Cantor, 1839) is one of the most common venomous snakes in southeastern Asia including China, northeastern Bangladesh, Vietnam, Myanmar and northeastern India (Gumprecht et al., 2004). This snake is frequently found in bamboo grove, bushwood, and farmland (Zhao, 2006). In China, this species exhibits a wide distribution ranging from Sichuan in the West to Taiwan in the East, from Hainan in the South to Shanxi in the North (Zhao, 2006). Some previous studies indicated that morphological investigation on widely distributed snakes could provide valuable data in detecting cryptic diversity for these mysterious animals (Devitt et al., 2008; Vogel et al., 2004). A wide distribution makes P. mucrosquamatus to be an ideal model to study SD and GV, and especially to explore whether cryptic diversity is present between mainland China and Hainan Island populations.

## 2. Materials and Methods

A suit of 29 characters including meristic, mensural, and marking (Appendix 1) were recorded from 128 specimens

<sup>&</sup>lt;sup>1</sup> College of Tourism and Urban-Rural Planning, Chengdu University of Technology, Chengdu 610059, China

<sup>&</sup>lt;sup>2</sup> College of Life Sciences and Food Engineering, Yibin University, Yibin 644007, China

<sup>\*</sup> Corresponding author: Prof. Peng GUO, from the College of Life Science and Food Engineering, Yibin University, Sichuan, China, with his research focusing on herpetology, particularly molecular systematics, morphology evolution, biogeography and population genetics of Asian reptiles.

collected from the Mainland China (Figure 1; Appendix 2). Measurements of body and tail lengths were taken with a ruler to the nearest 1 mm; the others were taken with a slide caliper to the nearest 0.1 mm. Symmetric head characters were taken only on the right side unless they were unavailable (e.g. damaged); meristic characters were recorded on both sides and the average was used in the analysis. Ventral scales were counted according to Dowling (1951). The number of dorsal scale rows were given at one head-length posterior to the head, mid-body (corresponding to half of the total ventral number), and one head-length anterior to the vent. Values for symmetric head characters are given in left/right order. All data were collected by the senior author to avoid inter-observer bias.

All morphometric analyses were carried out using SPSS 20.0 (SPSS Inc., Chicago), and statistical tests were considered to be significant at  $P \leq 0.05$ . Males and females were analyzed separately. Missing values were replaced with the group mean (for non-allometric characters). For allometric characters, they were adjusted using the pooled within-group regression coefficient against either snout-vent length (SVL) or head length (LHEAD) to remove size-related bias. Missing values were filled by using group mean for characters in which less than 30% of specimens in that group had missing values. If more than 30% character data missing for a given specimen, then the specimen was excluded

in subsequent analyses. Characters were checked for significant between-group variation by using a one-way analysis of variance (ANOVA) and co-variance (ANCOVA). Non-significant characters were not used in subsequent analyses. A few characters were highly correlated with each other (r > 0.7), indicating that they may result in over-emphasis of the correlated variables in PCA (Thorpe, 1976). Thus, only one of the characters from the correlated character sets was used. Principal component analysis (PCA) was implemented on the characters showing significant between-group and between-sex differences to explore and describe variation pattern of the morphological data.

## 3. Results

Morphometric analyses revealed that eight of 29 characters were different between sexes and seven were significantly different (Table 1). For example, females exhibit more VS than males, while males have more SC, longer tail and bigger eye than females (Table 1). Simple linear regression analyses based on WHEAD and LHEAD displayed that slope of the females was stepper than that of males (Figure 2). Based on residual scores from a linear regression of head size on SVL, females were significantly larger than males in LHEAD and WHEAD (Table 1).

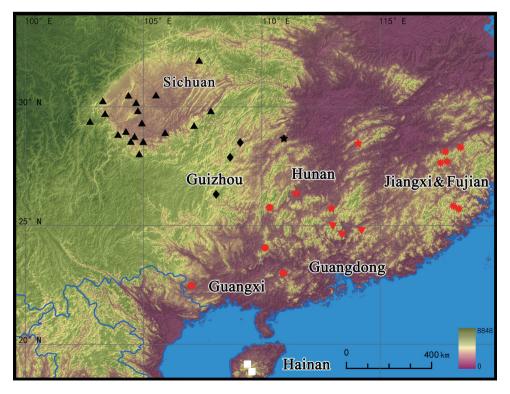


Figure 1 The localities of P. mucrosquamatus specimens examined.

A one-way ANOVA revealed that eight characters, including NUINT, SUBO, SUP, BSUP, POS, SUBL, NUTCHI, ALIS were not significantly different between groups, these characters were thus not used in subsequent analyses. PCA results showed that P. mucrosquamatus formed two distinct clusters in males. The first cluster includes all specimens from Hainan Island, and the second is composed of the other specimens (Figure 3). The first two components explain a cumulative variance of 64.95%, and component 1 explains a higher cumulative variance than component 2 (41.09% vs. 23.86%). Component 1 is dominated mainly by VS, SC, SC8to6, and SC6to4, and component 2 is dominated by NUS. The cluster 2 which included most of specimens examined did not show a distinct geographic structure, and most specimens from different localities overlapped in multivariate phenetic space and could not be separated from each other. Analyses of the females did not show

distinct clusters as the males (Figure 3).

Another ANOVA analysis, which was conducted for males for the two clusters detected, showed that four characters (VS, SC, SC8to6, SC6to4) were significantly different (Table 2). The specimens from Hainan Island have more VS and SC than the others, as well as the more posterior occurrence of the subcaudal scale reduction from 8 to 6 (SC8to6) and from 6 to 4 (SC6to4) than the others (Table 2). However, the females from Hainan Island and mailand China were not detected significant difference between clusters.

## 4. Discussion

**4.1 Sexual dimorphism** Sexual dimorphism (SD) is the condition where two sexes of the same species exhibit different characteristics including size, color, markings, and also behavioral differences. These differences may

**Table 1** Statistical summary of morphological characters showing sexual dimorphism in *P. mucrosquamatus*. Size-related characters are adjusted to a common size of SVL by ANCOVA; WHEAD and LHEAD used residual scores from a linear regression on SVL.

Characters	Numbers	Minimum	Maximum	$Mean \pm SD$	F	Р
SVL(M/F)	56/72	61.5/48.5	101.9/101.3	80.58 ± 10.38 / 77.68 ± 10.78	2.364	0.127
SC (M/F)	56/72	75/67	100/88	$87.73 \pm 4.87 / 79.72 \pm 3.57$	115.36	0
VS (M/F)	56/72	193/198	219/225	$204.98 \pm 6.04 / 210.72 \pm 5.13$	33.73	0
TL (M/F)	56/72	14.3/9.90	28.1/22.5	$19.76 \pm 3.22 / 16.33 \pm 2.67$	120.41	0
DIAE (M/F)	56/72	3.99/3.59	5.65/5.72	$4.81 \pm 0.42 / 4.48 \pm 0.47$	22.26	0
SC10to8 (M/F)	48/61	10.5/5.75	28/21.5	$20.07 \pm 3.66 / 14.40 \pm 3.38$	70.213	0
SC8to6 (M/F)	49/60	22.5/18.5	48.25/41.75	$35.83 \pm 6.36 / 29 \pm 5.34$	37.15	0
WHEAD (M/F)	56/72	-5.53/-5.11	2.83/8.05	$-0.52 \pm 1.85 / 0.41 \pm 2.20$	6.40	0.013
LHEAD (M/F)	56/72	-4.17/-3.93	3.79/10.16	$-0.71 \pm 1.63 / 0.55 \pm 2.08$	13.91	0

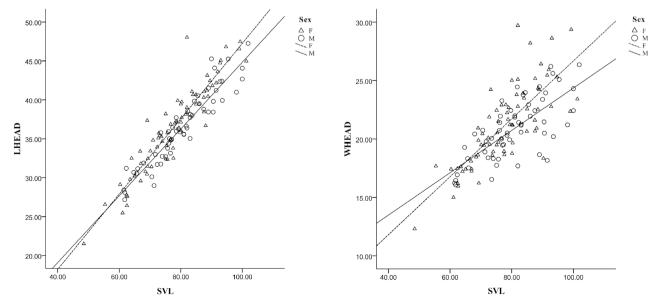


Figure 2 Regressions of head width (WHEAD) and head length (LHEAD) on Snout-vent length (SVL) for *P. mucrosquamatus*. Slope differs between WHEAD (F>M) and LHEAD (F>M).

be subtle or exaggerated. The condition occurs in many animals, insects, birds, reptiles, and even some plants. A growing body of literature reported the presence of SD in snakes (e.g. Manier, 2004; Shine, 1978). Our present study on P. mucrosquamatus revealed that this species also exhibit SD, and SD was displayed only in several characters (e.g. SC, VS, TL, DIAE, SC10to8, SC8to6, LHEAD, and WHEAD). Males of P. mucrosquamatus have longer tail than females, which was similar to an Asian pit viper Viridovipera stejnegeri (Lin, 2004) and some other colubrids such as *Xenochrophis piscator*, Rhabdophis tigrinus, Sinonatrix percarinata (Lei, 2005), and Thamnophis sirtalis (Shine, 1999). King (1989) and Shine (1993) proposed that long tail was common in male snakes. Shine (1999) confirmed that long tail has long hemipenis, and partial tail loss led to reduce the rate of mating success, thus the tail differentiation between sexes may be the result of sex-selection. For head shape, female P. mucrosquamatus have lager LHEAD and WHEAD than males, which was similar to Laticauda colubrina (Shine, 2002). Vincent et al. (2004) advocated that head size differentiation between sexes reflected the functional differences on predation, thus reduce the resource competition between sexes. The head size differentiation

between sexes may be resulted from nature-selection.

**4.2 Geographic variation** Geographic variation (GV) of species is determined by geographic variables such as climate or ecological niche as well as population genetic factors. PCA analyses revealed significant geographic variation between the population from Hainan Island and mainland China in male P. mucrosquamatus, while the same result was not detected in females. In males. Hainanese P. mucrosquamatus generally have more SC and VS than that in mainland China, and subcaudal scale reduction from eight to six, and from six to four occurs in more posterior than that from mainland China. Geographical variation in morphological characters sometimes features distinct subspecies or species. Although Hainan population is significantly different from the mainland population in several scalation characters (based on mean) in males, these characteristics are gradual or weakly overlapped between these two groups (addition of specimens from Hainan may reveal strong overlap) (Table 2). Recently, phylogeographic analysis based on mtDNA revealed that the Hainan population was a distinct lineage, however some hypothesis tests consistently rejected the Hainan population was a different taxon (Guo et al., in press.). Thus based on an

**Table 2** Morphological characters showing significant difference between populations from Hainan Island and mainland China in male *P. mucrosquamatus*.

Characters -	Hainan Island			Mainland			
	mean	minimum	maximun	mean	minimum	maximun	r
VS	216.25	214	219	204.12	193	215	0
SC	97	94	100	86.86	75	94	0
SC8to6	44.56	41.5	46.5	34.79	22.5	48.25	0.004
SC6to4	80.69	77	85	65.72	52	77.5	0

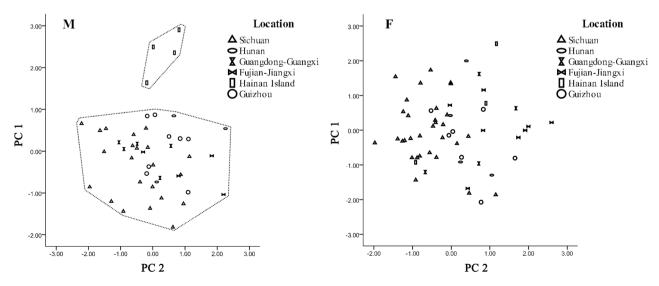


Figure 3 Plots of the first two principal components for male (left) and female (right) P. mucrosquamatus.

integrative taxonomic approach advocated by Torstrom *et al.* (2014), we proposed that *P. mucrosquamatus* was conservative in external morphology, and no significantly intraspecific differentiation was present. A similar case was explored recently in its co-distributed Asian pit viper *Viridovipera stejnegeri* (Guo *et al.*, 2016).

Acknowledgements This project was funded by the National Natural Science Foundation of China (NSFC31372152) and the Scientific Research Fund of Sichuan Provincial Education Department (13TD0027) to Peng Guo. We thank Chengdu Institute of Biology, the Chinese Academy of Sciences for permission for specimen examination, and we also extend our thanks to Dr. Jia-Tang Li (CIB) for his assist in specimen examination.

## References

- **Aubret F., Maumelat S.** 2005. Sex differences in body size and ectoparasite load in the Ball Python, *Python regius*. J Herpetol, 39(2): 315–320
- Bonnet X., Shine R., Naulleau G., Vachervallas M. 1998. Sexual dimorphism in snakes: Different reproductive roles favour different body plans. P Roy Soc Lond B-Bio Sci, 265(1392): 179–183
- Cantor T. E. 1839. Spicilegium serpentium indicorum [part 1]. Proc Zool Soc Lond, 1839: 3–34
- **Campbell B.** 1972. Sexual selection and the descent of man 1871–1971. Chicago: Aldine
- **Dowling H. G.** 1951. A proposed standard system of counting ventrals in snakes. Br J Herpetol, 1: 97–99
- Darwin C. 1871. The descent of man, and selection in relation to sex. London. UK: John Murray
- Devitt T. J, LaDuc T. J, McGuire J. A. 2008. The *Trimorphodon biscutatus* (Squamata: Colubridae) species complex revisited: A multivariate statistical analysis of geographic variation. Copeia, 2: 370–387
- **Gregory P. T.** 2004. Sexual dimorphism and allometric size variation in a population of Grass Snakes (*Natrix natrix*) in Southern England. J Herpetol, 38(2): 231–240
- Gumprecht A., Tillack F., Orlov N. L., Captain A., Ryabow S. 2004. Asian pitvipers. Berlin: Geitje Books
- Guo P., Liu Q., Zhu F., Zhong G. H., Chen X., Myers E. D., Che J., Zhang L., Ziegler T., Nguyen T. Q., Burbrink F. T. 2016. Complex longitudinal diversification across South China and Vietnam in Stejneger's pit viper, Viridovipera stejnegeri

- (Schmidt, 1925) (Reptilia: Serpentes: Viperidae). Mol Ecol, 25: 2920–2936
- **King R. B.** 1989. Sexual dimorphism in snake tail length: sexual selection, natural selection, or morphological constraint? Biol J Linn Soc, 38: 133–154
- Lin Z. H., Lei H. Z. 2004. Sexual dimorphism and interspecific differentiation of tail length in five adders (Viperidae). Henan Sci, 22(6): 789–791 (In Chinese)
- Lei, H. Z., Lin, Z. H., Hua, H. L. 2005. Sexual dimorphism and interspecific differentiation of tail length in six snakes of racer (*Natrix*). Henan Sci, 23(2): 211–213 (In Chinese)
- **Malhotra A., Thorpe R. S.** 2004. Maximising information in systematic revisions: a combined molecular and morphological analysis of a cryptic green pitviper complex (*Trimeresurus stejnegeri*). Biol J Linn Soc, 82: 219–235
- Manier M. K. 2004. Geographic variation in the long–nosed snake, *Rhinocheilus lecontei* (Colubridae): beyond the subspecies debate. Biol J Linn Soc, 83(1): 65–85
- **Shine R. G.** 1978. Sexual size dimorphism and male combat in snakes. Oecologia, 33: 269–277
- Shine R. G. 1986. Sexual difference in morphology and niche utilization in an aquatic snake, *Acrochordus arafurae*. Oecologia, 69: 260–267
- **Shine R. G.** 1993. Sexual dimorphism in snakes. pp. 49–86. In Seigel R. A., Collins J. T. (Eds.), Snakes: Ecology and Behavior. New York: McGraw-Hill
- Shine R. G. 1994. Sexual size dimorphism in snakes revisited. Copeia: 326–346
- Shine R. G., Olsson M. M., Moore I. T., LeMaster M. P., Mason R. T. 1999. Why do male snakes have longer tails than females? P Roy Soc Lond B-Bio Sci, 266: 2147–2151
- **Shine R. G., Reed R. N., Shetty S., Cogger H. G.** 2002. Relationships between sexual dimorphism and niche partitioning within a clade of sea-snakes (Laticaudinae). Popul Ecol, 133: 45–53
- **Thorpe R. S.** 1976. Biometric analysis of geographic variation and racial affinities. Biol Rev, 51: 407–452
- Torstrom S. M., Pangle K. L., Swanson B. J. 2014. Shedding subspecies: The influence of genetics on reptile subspecies Taxonomy. Mol Phylogenet Evol, 76: 134–143
- Vincent S. E, Herrel A., Irschick D. J. 2004. Sexual dimorphism in head shape and diet in the cottonmouth snake (*Agkistrodon piscivorus*). J Zool, 264(1): 53–59
- Vogel G., Pauwels O. S. G., David P., Brachtel N. 2004. On the occurrence of the water snake *Sinonatrix aequifasciata* (Barbour, 1908) (Serpentes, Colubridae, Natricinae) in Vietnam. Hamadryad, 29(1): 110–114
- **Zhao E. M.** 2006. Snakes of China. Hefei: Anhui Science and Technology Publishing House. 1–369(In Chinese)

**Appendix 1** A list of morphological characters recorded in this study. The characters unsatisfied the normal distribution are indicated by asterisks. More detailed description of head characters is shown in Appendix 3.

Measurement	Abbr.
Snout-vent length	SVL
Tail length	TL
Head width	WHEAD
Head length	LHEAD
The length of the mouth	ML
The length of the supraocular	LSUP
The diameter of the eye	DIAE
The distance between supraocular and middle of the rostral	DISSR
The distance between anterior of nostril and anterior of eyes	DISNE
The width between outside of supraocular	WIDSUP
Number of ventral scales	VS
Number of subcaudal scales	SC
Dorsal	DOR*
Supralabial	SUP*
Sublabials	SUBL*
Subocular	SUBO*
Postoculars	POS*
Number of small scales between internasals	NUINT*
Number of scales between supraoculars	NUSUP*
Numbers of sublabial touching with anterior chin-shield	NUTCHI*
The biggest supralabial	BSUP*
The spot on back (Bilateral average)	NUS
Aligned spot on back	ALIS
Ventral scale position of the reduction from 25 to 23 scale rows	VS25to23*
Ventral scale position of the reduction from 21 to 19 scale rows	VS21to19*
Ventral scale position of the reduction from 23 to 21 scale rows	VS23to21*
Subcaudal scales position of the reduction from 10 to 8 scale rows	SC10to8*
Subcaudal scales position of the reduction from 8 to 6 scale rows	SC8to6
Subcaudal scales position of the reduction from 6 to 4 scale rows	SC6to4

**Appendix 2** Specimens examined in this study. CIB: Chengdu Institute of Biology, the Chinese Academy of Sciences; YBU: Yibin University.

# Sichuan-Chongqing:

Junlian: YBU12179, YBU091108, YBU091109, YBU12092A, YBU13060, YBU13061; Changning: YBU081014; Hejiang: YBU12228, YBU091058, YBU071084, YBU071081, YBU091059, YBU091055, YBU091056; Zizhong: YBU12041, YBU12042, YBU12043, YBU12046, YBU12089, YBU12039; Zigong: YBU12037, YBU12086, YBU12088, YBU12087, YBU12034, YBU091114, YBU12040, YBU12036, YBU12035; Yibin: YBU071060, YBU071090, YBU071061, YBU091024, YBU071062, YBU12131; Shimian: CIB72562, CIB13548, CIB13549; Guang'an: YBU13066; Longquan: YBU11008, YBU11009, YBU11007, YBU11004; Emei: CIB13567, CIB13525, CIB13526, CIB101204, CIB101203, CIB13533, CIB13529, CIB13528, CIB13527, CIB13520, CIB13532; Ya'an: YBU12123, YBU11297, YBU11296, YBU12122, YBU11298, YBU10041, YBU12129; Sichuan(no detail locality): YBU051003, YBU051005, YBU051007; Nanchuan: YBU0911350; Xiushan: YBU11269, YBU11262; Fengdu: CIB13554.

#### Guizhou:

Pingyang: YBU12199, YBU12182, YBU12183, YBU12196, YBU12200, YBU12201, YBU12202, YBU12198, YBU12204, YBU12187, YBU12205, YBU12188, YBU12197; Tongren: YBU13244.

### Hainan Island:

Lingshui: CIB95499, CIB13559, CIB95498, CIB13546; Baisha: CIB13560; Wuzhishan: CIB78110, CIB78578, CIB78576.

## Guangdong-Guangxi

Ruyuan: YBU12107, YBU071045, YBU071055; Shixing: YBU11046, YBU13108, YBU11045; Guilin: YBU061002; Cenxi: YBU091078; Jinxiu: YBU061001, CIB13565, CIB13561, CIB13508; Guangxi (no detail locality): CIB13563, CIB13564, CIB13562.

## Jiangxi-Fujian:

Dehua: CIB13514, CIB13516, YBU12161, CIB13515, YBU12160, CIB13518; Wuyishan (=Chong'an): CIB13512, CIB13519, CIB13513; Jianyang: CIB13511; Shangrao: CIB13557, CIB13555; no detail locality: CIB13517.

#### Hunans

Yongzhou: YBU11015, YBU11018, YBU11016, YBU11014; Liuyang: YBU13289; Yizhang: CIB13551, CIB13552, CIB13550; Yuanling: YBU11272; no detail locality: YBU11273.

**Appendix 3** Explanation of some head characters relating to scalation and measurement in *P. mucrosquamatus*. Lateral (upper) and dorsal views (lower).

